AD_____

Award Number: DAMD17-99-1-9406

TITLE: hRAD51 Involvement in Genomic Instability and Development of Breast Cancer

PRINCIPAL INVESTIGATOR: Richard A. Fishel, Ph.D.

CONTRACTING ORGANIZATION: Thomas Jefferson University
Philadelphia, Pennsylvania 19107

REPORT DATE: September 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2000	Annual (23 Aug 99 - 24 Aug 00)

4. TITLE AND SUBTITLE
hRAD51 Involvement in Genomic Instability and Development of Breast Cancer

5. FUNDING NUMBERS
DAMD17-99-1-9406

6. AUTHOR(S)
Richard A. Fishel, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Thomas Jefferson University
Philadelphia, Pennsylvania 19107

E-MAIL:
Rick.Fishel@mail.tju.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

It becomes increasingly clear that both BRCA1 and BRCA2 tumor suppressor genes are involved in hRAD51-dependent DNA repair by homologous recombination. In this study, we will test whether DNA recombinational repair may be compromized during breast cancer development. We first examined the hRAD51 gene in tumors with 15q14-15 deletions. We did not find any changes compared to normal tissue which supports (among other possibilities, see accompanying reprint) the notion that hRAD51 is an essential gene.
We have also examined interactions among members of the hRAD51 protein family and between hRAD51 family members and BRCA1/2. All hRAD51 homologs seem to exist in a multiprotein complex (hRAD51 proteome), and examination of the functional significance of these interactions is currently in progress.

14. SUBJECT TERMS
Breast Cancer, hRAD51 homologs, DNA recombination, BRCA1, BRCA2

15. NUMBER OF PAGES
14

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Cover.....1

SF 298.....2

Table of Contents.....3

Introduction.....4

Body.....4

Key Research Accomplishments.....5

Reportable Outcomes.....6

Conclusions.....6

References.....7

Appendices.....8

(4) INTRODUCTION:

Multiple mutations and changing gene expression patterns occur during cancer development, and a mutator phenotype has been proposed to be a prerequisites for carcinogenesis (1). Both DNA repair and check point control can be understood as mechanisms to safeguard the integrity of the genome, and the majority of tumor suppressors discovered so far fall into these categories. In analogy to the importance of DNA mismatch repair for the development of gastrointestinal tumors, especially HNPCC, DNA recombinational repair dependent on human RAD52 epistasis group genes including RAD51 and its homologs might play a role in preventing wide-spread genomic instability leading to breast cancer. Evidence for the involvement of both BRCA1 and BRCA2 in hRAD51-mediated repair processes is accumulating (2-4). We test this hypothesis by examining the hRAD51 family members in human tumor samples and by characterizing structural and functional interactions among hRAD51 homologs (hRAD51 proteome) and between these proteins and BRCA1/2, respectively.

(5) BODY

a) Analysis of hRAD51 gene in tumor tissues. During the first year of the funded period, we focused on the examination of the hRAD51 gene in tumor tissues (Aim I). We sequenced the hRAD51 coding region in human tumors with high frequency of deletions (loss of heterozygosity) at the hRAD51 genomic locus (15q14-15). No changes were found suggesting that hRAD51 is not a tumor suppressor because it is either an essential gene, redundant gene and/or independent of the BRCA1/BRCA2 tumor suppressor pathway (see attached reprint: *Cancer Res.* 59, 4564, 1999).

b) Cloning and characterization of interactions between hRAD51 and its homologs. Members of the human RAD52 epistasis group like the hRAD51 homologs hRAD51B (also known as hRAD51L1 or hRAD51-H2), hRAD51C, hRAD51D (a.k.a. hRAD51-H3), XRCC2 and XRCC3 in addition to hRAD52 and hRAD54 were cloned into appropriate expression vectors. This collection allowed us to clearly identify static interactions between these six human mitotic RecA homologs, hRAD52, hRAD54 and BRCA2 which suggest a higher order complex (Aim IIIa; Figure 1). Interaction studies were done using an *in vitro* GST-fusion.IVTT method as described (5, 6). We found strong interactions between hRAD51B and hRAD51C, hRAD51D and

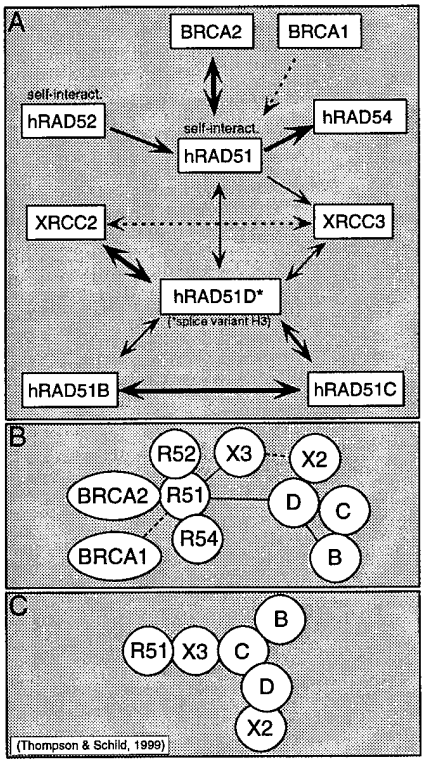


Figure 1. Summary of hRAD51 Proteome. Arrows indicate substantial GST-fusion interaction with IVTT substrate. Two-way arrows indicate reciprocal interactions; one-way arrows indicate interaction detected uni-directionally. Panel A and B: Interactions and model using GST-fusion/IVTT method; Panel C: Interactions according to Thompson and Schild (4).

XRCC2, hRDA51C and hRAD51D, while weaker interactions were found between hRAD51 and XRCC3, hDRAD51 and hRAD51D, and hRAD51B and hRAD51D, respectively. Based on two-hybrid studies, an interaction map has also been developed for the mitotic RecA homologs (7). However, our interaction data appear to differ significantly with these studies (Figure 1). For example, we find that hRAD51D appears to occupy a central position in the interaction with most other mitotic RecA homologs (Figure 1B), while Thompson and Schild (7) suggest that hRAD51C plays a central role in interacting with 3 of 6 mitotic RecA homologs (Figure 1C). These differences may be the result of the different systems used. However, it is interesting to note that we have confirmed reciprocal interactions for all of the mitotic RecA homolog GST-fusion/IVTT associations.

To verify these interaction results, we overexpressed the hRAD51 derivatives and produced specific antibodies, which will be very useful in co-immunoprecipitation experiments.

c) Purification and characterization of XRCC2. Since all hRAD51 derivatives (with the exception of hRAD51) appear to be largely insoluble when expressed in bacteria, we purified XRCC2 using a baculovirus expression system (Aim III; Figure 2). Biochemical studies to characterize this protein and its possible effects on hRAD51 function and purification of the remaining homologs are currently in progress.

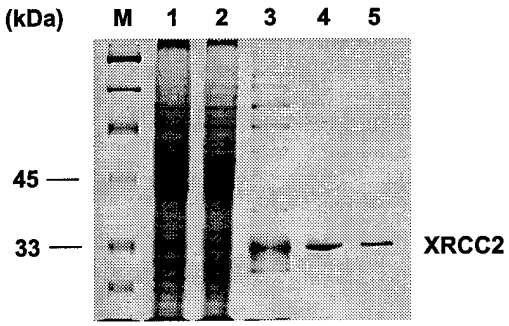


Figure 2. Purification of XRCC2. M: molecular weight marker, lanes 1-5: cellular lysate and relevant fractions obtained during purification by affinity chromatography.

(6) KEY RESEARCH ACCOMPLISHMENTS:

- The coding region of the hRAD51 gene has been examined for mutations in tumor tissues with high frequencies of 15q15 deletions, and the promoter region has been tested for hypermethylation (Aim I). No changes have been found in the tumors compared to normal tissues.

- All known human RAD51 homologs involved in mitotic recombination have been cloned into appropriate vectors and overexpressed in bacteria (Aim II). Polyclonal antibodies against these proteins have been generated and characterized.

- Previously unknown chromosomal locations of RAD51 homologs have been determined (Aim IIa).

• Promoter regions of hRAD51D and XRCC2 have been cloned (Aim IIb), and further characterization of these sequences is in progress.

• All major interactions between hRAD51 and its homologs have been determined (Figure 1; Aim IIIa).

• As a first hRAD51 homolog, XRCC2 has been purified to near homogeneity using a baculovirus expression system (Figure 2, Aim IIIb), and we have begun the process of characterizing the effect(s) of XRCC2 on hRAD51 adenosine nucleotide-regulated activities which appear to be controlled by both the ADP→ATP exchange and ATP→ADP+P_I hydrolysis steps.

(7) REPORTABLE OUTCOMES:

• The results of the mutational analysis of the hRAD51 coding region have been published in:
Schmutte, C., Tombline, G., Rhiem, K., Sadoff, M. M., Schmutzler, R., von Deimling, A., and Fishel, R. (1999). Characterization of the Human Rad51 genomic locus and Mutational Analysis of Tumors with LOH at 15q14-15q15.1. Cancer Res 59, 4564-4569.

• Preliminary results about XRCC2/hRAD51 interactions have been presented at the DNA Repair and Mutagenesis Meeting of the American Society for Microbiology (November 1-7, 1999 in Hilton Head, SC).

• Antibodies against all six known hRAD51 homologs involved in mitotic recombination were generated and characterized.

(8) CONCLUSIONS

• No mutations or promoter methylation changes have been found in the hRAD51 gene in tumors with high frequencies of 15q14-15 deletions. It appears that hRAD51 is an essential protein for cellular proliferation, and other homologs may be more likely to be targets for inactivation during carcinogenesis (see also discussion of the accompanying reprint of *Cancer Res.* 59, 4564, 1999). It is interesting to note that mutations in primary cancers have been found in hRAD54 and hRAD54B, other members of the hRAD52 epistasis group, although at very low frequencies (8-10).

• Multiple interactions exist between hRAD51 and its homologs (Figure 1), and purification of these proteins is in progress in order to study the functional relevance of these interactions.

(9) REFERENCES

1. Loeb, L. A. Mutator phenotype may be required for multistage carcinogenesis, Cancer Res. *51:* 3075-3079, 1991.
2. Chen, C.-F., Chen, P.-L., Zhong, Q., Sharp, Z. D., and Lee, W.-H. Expression of BRC Repeats in Breast Cancer Cells Disrupts the BRCA2-Rad51 Complex and Leads to Radiation Hypersensitivity and Loss of G2/M Checkpoint Control, J. Biol. Chem. *274:* 32931-32935, 1999.
3. Yuan, S.-S. F., Lee, S.-Y., Chen, G., Song, M., Tomlinson, G. E., and Lee, E. Y.-H. P. BRCA2 Is Required for Ionizing Radiation-induced Assembly of Rad51 Complex in Vivo, Cancer Res. *59:* 3547-3551, 1999.
4. Moynahan, M. E., Chiu, J. W., Koller, B. H., and Jasin, M. Brca1 Controls Homology-Directed DNA Repair, Mol Cell. *4:* 511-518, 1999.
5. Guerrette, S., Wilson, T., Gradia, S., and Fishel, R. Interactions of Human hMSH2 with hMSH3 and hMSH2 with hMSH6: Examination of Mutations Found in Hereditary Nonpolyposis Colorectal Cancer, Mol Cell Biol. *18:* 6616-6623, 1998.
6. Schmutte, C., Marinescu, R. C., Sadoff, M. M., Guerrette, S., Overhauser, J., and Fishel, R. Human exonuclease I interacts with the mismatch repair protein hMSH2, Cancer Res. *58:* 4537-4542, 1998.
7. Thompson, L. H. and Schild, D. The contribution of homologous recombination in preserving genome integrity in mammalian cells, Biochimie. *81:* 87-105, 1999.
8. Hiramoto, T., Nakanishi, T., Sumiyoshi, T., Fukuda, T., Matsuura, S., Tauchi, H., Komatsu, K., Shibasaki, Y., Inui, H., Watatani, M., Yasutomi, M., Sumii, K., Kajiyama, G., Kamada, N., Miyagawa, K., and Kamiya, K. Mutations of a novel human RAD54 homologue, RAD54B, in primary cancer, Oncogene. *18:* 3422-3426, 1999.
9. Matsuda, M. M., Miyagawa, K., Takahashi, M., Fukuda, T., Kataoka, T., Asahara, T., Inui, H., Watatani, M., Yasutomi, M., Kamada, N., Dohi, K., and Kamiya, K. Mutations in the RAD54 recombination gene in primary cancers, Oncogene. *18:* 3427-3430, 1999.
10. Rasio, D., Murakumo, Y., Robbins, D., Roth, T., Silver, A., Negrini, M., Schmidt, C., Burczak, J., Fishel, R., and Croce, C. M. Characterization of the human homologue of RAD54: a gene located on chromosome 1p32 at a region of high loss of heterozygosity in breast tumors, Cancer Research. *57:* 2378-83, 1997.

(10) APPENDICES

• Reprint of Schmutte *et al.*, Characterization of the Human Rad51 genomic locus and Mutational Analysis of Tumors with LOH at 15q14-15q15.1. *Cancer Res.* 59, 4564-4569.

[CANCER RESEARCH 59, 4564–4569, September 15, 1999]

Advances in Brief

Characterization of the Human *Rad51* Genomic Locus and Examination of Tumors with 15q14–15 Loss of Heterozygosity (LOH)[1]

Christoph Schmutte,[2] Gregory Tombline,[2] Kerstin Rhiem, Margaret M. Sadoff, Rita Schmutzler, Andreas von Deimling, and Richard Fishel[3]

Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania 19107 [C. S., G. T., M. M. S., R. F.]; Universitäts-Frauenklinik, Universität Bonn, 53105 Bonn, Germany [K. R., R. S.]; and Universitätsklinikum Charité, Humboldt-Universität, Institut für Neuropathologie, D-13353 Berlin, Germany [A. v. D.]

Abstract

Human *Rad51* (*hRad51*) has been found to be associated with BRCA1, BRCA2, and p53 either directly or indirectly and is one of at least eight human genes that are members of the *Escherichia coli* RecA/*Saccharomyces cerevisiae* Rad51 family thought to affect genomic stability through DNA recombination/repair processes. While inactivation of DNA mismatch repair clearly leads to instability of repeated sequences and to an increased risk for tumorigenesis, such a parallel for the RecA family members has not been reported. Recently, a high frequency of loss of heterozygosity at chromosome 15q14–15, near the genomic region containing *hRad51*, has been reported in human tumors (W. Wick *et al.*, Oncogene, *12:* 973–978, 1996). To determine whether *hRad51* inactivation may be involved in the etiology of these tumors, we have characterized the *hRad51* genetic locus and mapped it to chromosome 15q14–15 within the central region of loss of heterozygosity. However, single-strand conformational polymorphism analysis and direct sequencing of tumors did not reveal any mutations in the *hRad51* coding sequence or intron/exon boundaries. We also examined the DNA methylation status of a CpG-rich region in the putative *hRad51* promoter region. No indication of hypermethylation was found. These results suggest that *hRad51* is not a tumor suppressor because it is either an essential gene, redundant gene and/or independent of the BRCA1/BRCA2 tumor suppressor pathway(s).

Introduction

Genomic instability is a common denominator in the vast majority of human cancers (1). The high number of genetic alterations found in tumor cells has led to the suggestion that an increased mutation rate (mutator phenotype), likely in combination with selection and clonal expansion, provides the requisite for tumorigenesis (2, 3). While up to 30% of colorectal tumors display instability in the length of repeated sequences (MSI;[4] Ref. 4), more than 70% of colorectal tumors exhibit gene amplifications and gross chromosomal changes that include chromosome translocations and alteration in chromosome numbers (aneuploidy; Ref. 5). Most MSI tumors harbor alteration(s) in the DNA mismatch repair genes (*hMSH2*, *hMLH1*, *hPMS2*, and *hMSH6*) that inactivate a pathway for repair of single-nucleotide and small insertion/deletion loop-type mismatches. In addition to MSI, inactivation of the mismatch repair machinery leads to an increase in spontaneous mutation rates, as would be predicted for a mutator phenotype (for recent reviews, see Refs. 6 and 7). A genetic basis for the 70% of colorectal tumors that exhibit gene amplifications and gross chromosomal changes has been suggested in experiments that demonstrate the persistence of the chromosomal aneuploidy phenotype (chromosomal instability) in colorectal cell lines (8). These studies support an aneuploidy mutator phenotype that is induced by as yet unidentified mechanisms.

Defects in recombination/repair pathways that process DNA damage, such as double-strand breaks, have been proposed to contribute to the observed genomic instability phenotype in some human cancers (1). Xeroderma pigmentosum, ataxia telangiectasia, Bloom's syndrome, Fanconi's anemia, and Nijmegen breakage syndrome are hereditary disorders in which chromosome breaks are known to contribute to an overall susceptibility to cancer (9, 10). Moreover, both the Bloom's syndrome and Nijmegen breakage syndrome gene products are related to bacterial or yeast homologues known to participate in the repair of broken chromosomes (11, 12).

The hRAD51 protein has been suggested to play a role as a tumor suppressor gene for several reasons: (*a*) hRAD51 is a member of the RecA/RAD51 family of proteins, which have been implicated in DNA recombination/repair processes (13); (*b*) the hRAD51 protein appears to associate either directly or indirectly with p53 (14), BRCA1 (15), and BRCA2 (16), three other known tumor suppressors; (*c*) RAD51 has been shown to function in *Saccharomyces cerevisiae* mutation avoidance (17), and the frequency and spectrum of a *rfa-1* mutant (the large subunit of the consensus cellular single-stranded binding protein) are enhanced by a *rad51* null mutation (18); and (*d*) the distribution of hRAD51 appears to be altered in response to DNA damage as well as along paired meiotic chromosomes in spermatocytes derived from $ATM^{-/-}$ mice (15, 19). Moreover, c-abl, an oncogene and downstream effector in the ATM DNA damage response pathway (20, 21), has been shown to phosphorylate hRAD51 and alter its ability to bind DNA *in vitro* (22). These results have led to the proposal that hRAD51 dysfunction in somatic cells might also contribute to genomic instability and cancer.

While both *Escherichia coli* RecA and the *S. cerevisiae* RAD51 proteins are not required for viability, mice containing a homozygous disruption of the *Rad51* gene display early embryonic lethality (23), and attempts to generate null *rad51* cell lines have failed (24). These findings would appear to limit a possible role for *hRad51* as a tumor suppressor gene. Interestingly, mammalian cells contain at least eight additional RAD51 homologues that may provide some redundancy in function or functional interaction. For example, it has been shown that XRCC3, one of these RAD51 homologues, interacts with hRAD51, and XRCC3 mutant cells display radiation sensitivity and increased chromosome aberrations (25, 26).

Here, we have characterized the *hRad51* genomic locus by defining the intron/exon boundaries and genetically mapping it relative to markers used previously in LOH studies of human tumors. We found that *hRad51* was located in the center of a region at chromosome 15q14–15, which is frequently deleted in breast tumors and metastatic brain tumors (27). We examined a cohort of these tumors that displays

Received 4/14/99; accepted 8/2/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by Grant CA56542 (to R. F.) and Grant DFG Schm 800/2-5 (to K. R.).

[2] C. S. and G. T. contributed equally to this work.

[3] To whom requests for reprints should be addressed, at Kimmel Cancer Center BLSB933, 233 South 10th Street, Philadelphia, PA 19107. Phone: (213) 503-1346; Fax: (215) 923-1098; E-mail: rfishel@hendrix.jci.tju.edu.

[4] The abbreviations used are: MSI, microsatellite instability; LOH, loss of heterozygosity; BAC, bacterial artificial chromosome.

Table 1 *Primers used for mutational analysis*

Exon 2	
CAAGCCCCTTATTTCTCTAGT	R37; 5′
CCTTCCACTAGGTAGAAGAA	R38; 3′
Exon 3	
GGACACATAACATCTGTGTTAG	R39; 5′
TGTACTATTCCCACTAATGCCT	R40; 3′
Exon 4	
TCAAGATCACTGTGGTAAGGA	R41; 5′
GCTTTCCTAACTAGAGTTCAC	R42; 3′
Exon 5	
CCAAGAACATTTCTATGACTACAG	R43; 5′
CAGGAATGAAGTAATGCTTGC	R44; 3′
Exon 6	
CTTGGTCAGCTGTATCAGAAAT	R45; 5′
GATAAGTGTAGCCATAGTCTCT	R45; 3′
Exon 7	
AGTTCTGTGTGCAGCCTAAA	R47; 5′
GGGAAGGACTCTTAAGAACAT	R48; 3′
Exon 8	
ACAGGCTAGAAATAGGCTTCA	R49; 5′
CTGAAAGTAGGCATTCTCTGT	R50; 3′
Exon 9	
GTCTATGGCCACAAAATTGACA	R51; 5′
TCCGAAAAGAAGAACTGATCC	R52; 3′
Exon 10	
CAAAGTCAGGAACGGAATTGT	R53; 5′
GCACTTAAGGTTTTTAACAGAGG	R54; 3′

LOH at 15q14–15 for *hRad51* mutations and promoter methylation. While we found no alterations of the *hRAD51* locus in the DNA derived from any of these human tumors, this study provides the tools necessary to further understand the regulation of *hRAD51* expression and to study its potential role in tumorigenesis.

Materials and Methods

Determination of *hRad51* Genomic Structure. Oligonucleotide primers specific for *hRad51* (R11, 5′-AGTGCTGCAGCCTAATGAGAGT-3′; and R12, 5′-TGTGACTACTGACCTGTCTCCT-3′) were used to screen a human BAC library (Version IV; Research Genetics, Huntsville, AL) by PCR. DNA from positive clones was isolated using the Qiagen (Chatsworth, CA) DNA purification kit. The DNA was sequenced on an Applied Biosystems automated sequencer, and intron-exon boundaries were determined by comparison with the cloned cDNA sequence as well as consensus splice site junctions. Intron sizes were confirmed by PCR followed by agarose gel electrophoresis. An additional 1.5 kb of 5′-untranslated sequence were sequenced and analyzed for potential transcription factor binding sites as well as the percentage of G+C bp and CpG sites.

Radiation Hybrid Analysis. The location of *hRad51* was determined using primers R11 and R12 to screen the Genebridge-4 radiation hybrid panel (Version RH02.02; Research Genetics; Huntsville, AL). PCR products were visualized by agarose gel electrophoresis, and data were submitted to the Whitehead Institute/MIT Center for Genome Research for interpretation. Using the same method, we determined the genomic position of marker GAAA1C11 (also known as D15S1232) using forward primer R33 (5′-CCAGAGAGATCTTTCCCCAT-3′) and reverse primer R34 (5′-TTGCTCCACTGTTTTCTCAG-3′), and we determined the genomic position of marker D15S641 using forward primer R31 (5′-AACAAAGGGAGACCCTCATC-3′) and reverse primer R32 (5′-GACACCCCAGTAGCAATGAG-3′). Information on the original characterization of these markers was obtained from the Cooperative Human Linkage Center.[5]

Tumor Samples. Native tumor specimens and corresponding normal blood were obtained from patients treated at the University Hospital-Bonn between 1990 and 1998 during surgery or at autopsy. Tissues were collected without bias for age, sex, or ethnic background. All tumors were classified according to the WHO guidelines. The tumor specimens were examined microscopically before phenol DNA extraction to exclude contamination by nontumorous tissue. DNA was isolated as described previously (27). Twenty brain metastases of tumors from various tissue origins and their normal counterparts were analyzed for mutations in the coding region of the *hRad51* gene. All tumors examined displayed LOH at 15q14–15 (Ref. 27; Table 2). A second set of 41 breast carcinomas (21 of which displayed LOH at 15q14–15) was examined for *hRad51* mutations (Table 2).

Single-Strand Conformational Polymorphism/Mutation Analysis. Details of the primer sets used to amplify the *hRad51* exons are shown in Table 1. Approximately 20 ng of genomic DNA were used as template in 20-μl reactions that contained 1.0 unit of AmpliTaq Gold (Perkin-Elmer); 200 μM each of dATP, dGTP, and dTTP; 40 μM dCTP; and 1.0 μCi of [α-^{32}P]dCTP. Cycles were as follows: 95°C for 12 min, followed by 30 cycles of 94°C for 30 s; 55.5°C for 30 s; 72°C for 45 s; followed by 5 min at 72°C. The reactions were heated at 94°C for 5 min and placed immediately on ice until loading. Samples were run at room temperature on 0.5× MDE gels (FMC BioProducts). Exons to be sequenced were amplified in a similar fashion, except that 200 μM dCTP was used. The PCR products were then isolated using agarose gel electrophoresis and the Qiagen Gel Extraction kit, and approximately 5 ng of PCR products were sequenced with an Applied Biosystems Automated Sequencer.

Methylation Analysis. Methylation of the predicted CpG island of the *hRad51* promoter was performed using the bisulfite method as originally described by Clark *et al.* (28). In brief, approximately 50 ng of DNA were digested with *Apa*I and then incubated with 2.5 M sodium metabisulfite (Amresco) and 100 mM hydroquinone (Sigma) at pH 5.0 in a total volume of 240 ml at 55°C for 16 h. Subsequently, the DNA was desalted, treated with 0.3 M NaOH at 37°C for 15 min, and precipitated. Primers were designed such that they flanked the region of highest CpG density as well as several Sp1 sites within the putative *hRad51* promoter upstream of exon 1 (see Fig. 3, ▩; 5′-TGAGGGATTGGGGTAGGAGTA-3′, 5′-CCAACCTTCTACACACAACCCAA-3′, 5′-GTAAAAAGGGAAGAGGGTAGTTTG-3′, 5′-ACAACCCAAATAAATTACAATTCCCAACT-3′). PCR products (274 bp) were synthesized by a nested PCR approach and sequenced directly. DNA treated with M.*Sss*I, which methylates CpG sites, were used as a positive control. There did not appear to be any differences in the amplification of methylated *versus* unmethylated substrate DNA. We estimate that 20% of methylated CpGs in the substrate DNA at most sites could be detected.

Results

Radiation Hybrid Mapping of the *hRAD51* Gene. The *hRAD51* gene was mapped previously by fluorescence *in situ* hybridization to chromosome 15q15.1 (29). This genomic locus is adjacent to a region (15q14) that has been reported to be frequently lost in brain metastases of various tumors (27). To test whether *hRad51* is involved in the development of these tumors, we first established the genomic location of the *hRad51* gene and many of the markers used in the tumor study relative to markers contained in a radiation hybrid panel (27). Radiation hybrid mapping revealed that *hRad51* is located between the most commonly deleted markers in these tumors, 26.7 cR telomeric from marker GAAA1C11 and 20 cR centromeric from marker D15S641 at 15q14–15 (Fig. 1). We also determined the location of nine polymorphic markers used to define the region of LOH on chromosome 15q14–15 in 21 metastatic brain tumors and 21 primary breast carcinomas. These data also indicate that the *hRad51* genomic locus is slightly more centromeric than reported previously but is clearly within the common region of LOH in both the brain and breast tumors.

Structure of the *hRad51* Genomic Locus. We determined the genomic structure of the *hRad51* gene to screen tumors for possible *hRad51* mutations. A BAC clone (21B18) containing the *hRad51* gene was identified by PCR screening of a human BAC DNA library (Research Genetics) using primers specific for *hRad51*. DNA from clone 21B18 was isolated and sequenced. Intron sizes were determined by long-range PCR (Boehringer Mannheim). PCR products could not be obtained for introns 5 and 6, suggesting intron sizes of >10 kb. The *hRad51* gene consists of 10 exons and spans at least 30 kb (Fig. 2*A*). All exon-intron boundaries follow the GT-AC rule. The translation start codon is located in exon 2 (Fig. 2*B*, *underlined*), and

[5] Address: http://www.chlc.org.

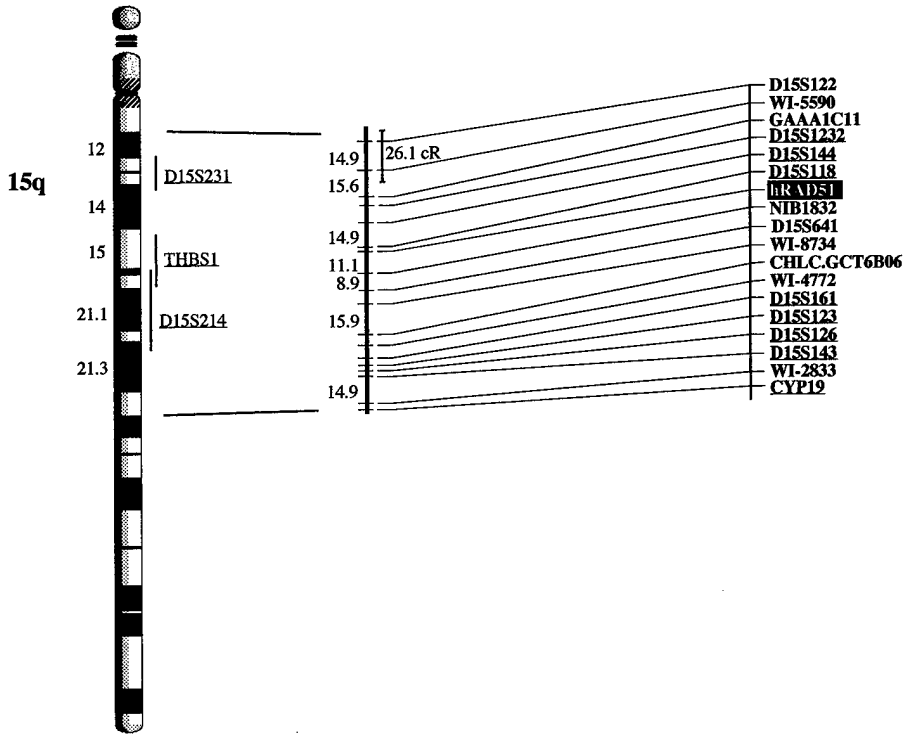
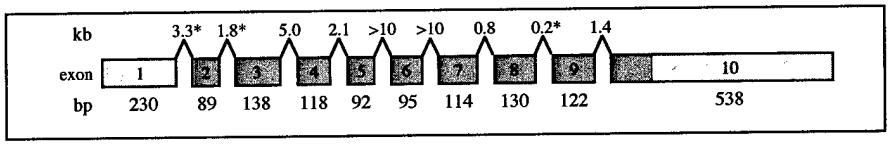


Fig. 1. Radiation hybrid analysis. The hRAD51 genomic locus, CYP19, and several additional microsatellite markers were mapped to chromosome 15q14–15 using the radiation hybrid method (see "Materials and Methods" for details). Markers used in LOH studies are *underlined*.

the average size of the coding exons is 112 bp. Further sequencing of the region 5′ of the first exon revealed that noncoding exon 1 contained a CpG island that was approximately 990 bp in size (Fig. 3). This putative promoter region contains several Sp1 recognition sites but lacks a TATA box. Further characterization of that region is in progress and will be published elsewhere. Sequences were submitted to GenBank (GenBank accession numbers AF165088, AF165089, AF165090, AF165091, AF165092, AF165093, and AF165094).

A.



B.

EXON 1

TTGGCGGGAATTCTGAAAGCCGCTGGCGGA-cc(1-230)ag-GTGAGTGTGTGAGGCGCAGGCTGGGCCCTCC

EXON 2

CTTATACTGATAAGCATTTGTATTTTTCAG-ta(231-319)ag-GTATGTGGTTAGTTGCTAATTTTGGAATTA

EXON 3

AAATTTATCCATGGTTTTCTTCATTTGCAG-ca(320-457)tg-GTAAGTACTGCTTACTTAACCTAGGGAGGCA

EXON 4

TGTTGATTTAATATTTCTTATTTTTCCCAG-gc(458-575)ag-GTGTAGTAATCCTTTATCCTGTGTTGTGAAC

EXON 5

GCTAAGAGTTATTTCTTATCGCTTTTTTAG-gt(576-667)ag-GTGAGCTGTTGGGGCTATAGCTAATCAAATA

EXON 6

CATTTCTACTGTTGTTTTTGTTCTCTATAG-ct(668-762)ag-GTAGGTTACTGGTTTAGATAAGAGAGACTAT

EXON 7

AGCCTAAAAATGTTCTCTCCTCTCTCATAG-gt(763-876)ag-GTATGTGTTCAGTATAAGACACCAAATATGTT

EXON 8

TAGGCTTCAGAGAATCCTTGTTTCCTGTAG-gt(877-1006)ag-GTAAGTTGTGGGATAGGGACAGAGAATGCCT

EXON 9

CAAAATTGACATTTATCCTTTCCCCATCAG-tt(1007-1128)ag-GTAAGGTGTTGATGGGATCAGTTCTTCTTTT

EXON 10

TTGGTGCTTTGGTCTGTGTCTTTGGGTCAG-at(1029-2229)tg-AATGT

Fig. 2. *hRad51* genomic structure. A human BAC clone containing the *hRad51* gene was identified by screening a BAC library (Research Genetics). Exon-intron borders were determined by direct sequencing of the BAC DNA. *A*, *hRad51* consists of 10 exons (*dark gray box*, coding exons; *light gray box*, noncoding exons) and covers ~30 kb (*, introns sequenced completely). *B*, exon-intron borders follow the GT-AG rule. *Capital letters*, intronic sequences. *Numbers in parentheses*, size (in bp) of the published cDNA sequence (GenBank accession number D14134).

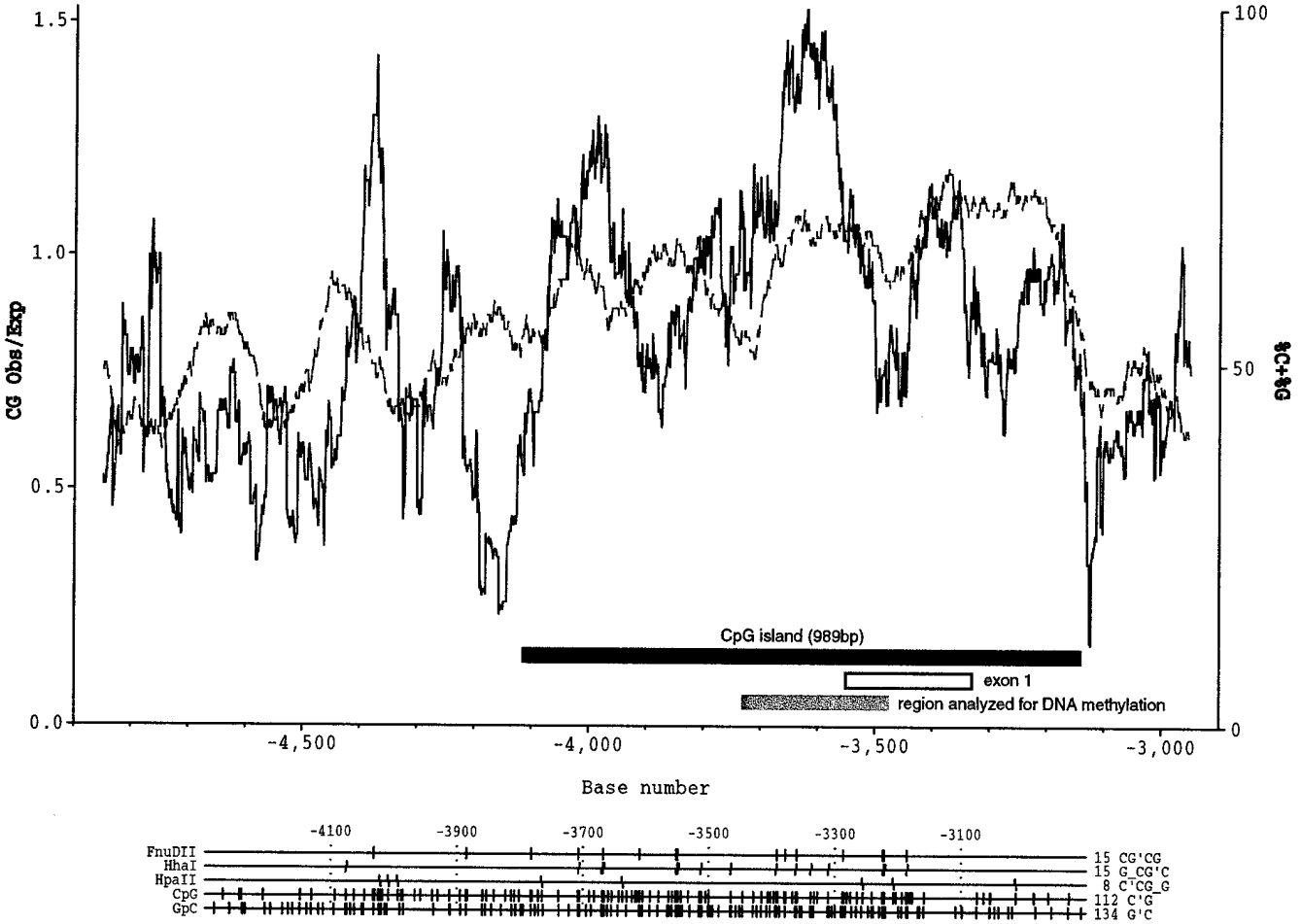


Fig. 3. Analysis of the CpG island in the 5′ region of the *hRad51* gene. The 5′ region of the *hRad51* gene was sequenced and analyzed using the GCG Software Package (GCG, Madison, WI). The translation start site (ATG) was used as 0. According to the rules established by Gardiner-Garden and Frommer (43), a region of 989 bp can be considered a CpG island (■), which covers exon 1 (□) and extends into intron 1. The region marked by the ▨ was examined for changes in DNA methylation.

Mutation Analysis of *hRAD51* in Human Tumors. All coding exons from 21 metastatic brain tumors and 42 breast carcinomas (including 21 that clearly displayed LOH at chromosome 15q14–15) were analyzed by the single-strand conformational polymorphism method using the primer pairs listed in Table 1. In addition, complete coding exons of 10 brain metastases were sequenced. No alterations in the coding sequence of *hRAD51* were detected (data not shown). These results suggest that nucleotide mutations of *hRAD51* that might lead to altered function of the hRAD51 protein were unlikely to be present in the cohorts of tumors that displayed LOH surrounding the *hRAD51* genetic locus.

Promoter Methylation Analysis in Human Tumors. We detected a large CpG island imbedded within the putative *hRad51* promoter region. CpG methylation may reduce or abolish expression of the *hRad51* gene, as has been shown for several tumor suppressor genes and repair genes including *hMLH1* (30) and the O^6-methylguanine methyltransferase gene (31). To rule out the possibility of both normal allele contamination and biallelic tumors, we examined promoter methylation in a region including the highest CpG density and several Sp1 binding sites in samples that clearly displayed LOH covering the *hRad51* genomic locus (Table 2; see dot samples). We found no evidence of promoter methylation in any of these samples in the region examined (data not shown), suggesting that *hRad51* expression is not impaired in tumors that display LOH at chromosome 15q14–15. These experiments must be tempered by the results of the bisulfite methylation detection method (28), in which there appeared to be a somewhat higher background between nucleotide −3693 and −3717).

Discussion

In this study we have: (*a*) characterized the structure of the *hRad51* genomic locus, which provided the tools necessary to specifically amplify *hRad51* coding regions from genomic DNA; and (*b*) tested the possibility that *hRad51* could function as a tumor suppressor gene in metastatic brain tumors and breast carcinomas that show high frequencies of LOH at chromosome 15q14–15.

Sequencing of the *hRad51* genomic locus revealed 10 exons, and a large (approximately 1 kb) CpG island covers the putative promoter region, which also includes the first (noncoding) exon. This CpG island appears TATA-less and is similar to typical housekeeping promoters (32). The presence of several putative Sp1 promoter binding sites is consistent with the observed cell cycle-dependent expression of *hRad51* (33). Previous reports have shown that both protein and mRNA levels rise at the G_1-S-phase boundary and remain elevated through the G_2-M phase (34). This G_1-S-phase pattern of expression is similar to that of yeast *Rad51* (35), possibly reflecting an S-phase-specific role other than recombination for RAD51 (36). Perhaps the most interesting difference between mammalian RAD51 and its yeast and bacterial family members is that its expression is not

Table 2 *Human tumor analysis*

SET1[a] ID	tumor	[b]	Microsatellite Marker[c] D15S165	D15S231	D15S1232	CYP19	D15S643
18	glioblastoma				LOH		
524	malignant peripheral nerve shealth tumor	•		LOH	h		
534	astrocytoma			LOH			
644	bronchial carcinoma metastasis	•		LOH	LOH		
662	bronchial carcinoma metastasis	•		LOH	ni		h
682	mamma carcinoma metastasis	•	LOH	LOH	LOH	LOH	LOH
722	lung carcinoma metastasis	•		LOH	ni	LOH	
730	oligodendroma			LOH	h		
732	lung carcinoma metastasis	•	LOH	LOH	LOH	LOH	LOH
764	glioblastoma				ni		
954	bronchial carcinoma metastasis	•		LOH	LOH	ni	
2006	bronchial carcinoma metastasis			LOH	LOH	LOH	
2124	lung adenocarcinoma metastasis	•	ni	LOH	LOH	h	
2194	rectal carcinoma metastasis	•		ni	LOH		
2240	gliosarcoma	•		ni	LOH		
2268	gliosarcoma	•		LOH	LOH		
2378	neurinoma	•		LOH	h		
2410	plexus papilloma			h	ni		
2468	medullablastoma			LOH		ni	
3786	oligoastrocytoma			ni			
3878	oligoastrocytoma			h			

hRad51

SET2[a] ID	[b]	Microsatellite Marker[c] D15S217	D15S144	D15S118	THBS1[d]	D15S214	D15S161	D15S123	D15S126	D15S143	CYP19
12		LOH	h	ni	h	ni	ni	LOH	h	h	h
42		h	ni	ni	ni	ni	ni	h	ni	h	LOH
62					ni						LOH
112		ni	h		ni	h	ni	h	ni	LOH	LOH
352	•	ni	LOH	ni	LOH	LOH	h	ni	LOH	LOH	ni
422	•	h	ni	ni	ni	ni	LOH	h	ni	ni	h
542		h	h	LOH	ni	h	ni	h	ni	h	h
762	•	h	ni	LOH	ni	LOH	ni	ni	ni	ni	ni
782		h	LOH?	ni	h	ni	ni	ni	ni	ni	LOH
882	•	ni	LOH	ni	ni	ni	h	ni	ni	ni	h
902		ni	ni	ni	h	h	ni	h	ni	h	LOH
952		ni	h	LOH	ni	h	h	h	LOH	h	ni
962	•	ni	ni	LOH	ni	LOH	ni	h	LOH	ni	ni
1072		ni	ni	h	ni	h	ni	h	ni	h	ni
1102		ni	LOH	LOH	ni	ni	h	ni	ni	ni	LOH
1112	•	LOH	ni	LOH	ni	LOH	ni	ni	h	LOH	LOH
1122	•	ni	ni	LOH	ni	ni	h	h	LOH	ni	LOH
1162					ni						LOH
1312		LOH	LOH	ni	ni	LOH	ni	LOH	LOH	LOH	LOH
1314			LOH	ni	ni	LOH	ni	LOH	LOH	LOH	LOH
1382		h	ni	ni	ni	h	ni	LOH	h	ni	h

hRad51

[a] Set 1, metastatic brain tumors; Set 2, primary breast tumors.
[b] Bisulfite methylation analysis of the region of the *hRad51* promoter described in Fig. 3.
[c] h, heterozygous; ni, not informative.
[d] The exact position of THBS1 relative to hRad51 has not been determined.

induced by DNA-damaging agents (37). Both the *E. coli* RecA and yeast RAD51 proteins are induced over 1000-fold during the SOS and RAD9 damage responses, respectively (38). While the mammalian p53 damage checkpoint/response has been compared to these pathways, we did not find p53 consensus transcription-activation sites in the *hRad51* promoter. Furthermore, recent reports suggest a p53/p21-dependent decrease in *hRad51* mRNA in response to ionizing irradiation (39). This distinction may classify *hRad51* more closely to genes such as PCNA, whose mRNA levels increase at the G_1-S-phase boundary but are down-regulated by p53 (40).

While hRAD51 mRNA levels are reduced in response to irradiation, proteins levels appear to persist (37), and hRAD51 becomes part of distinct nuclear foci (41). Other distinct nuclear foci of hRAD51 have been identified during S phase (36) and in B cells induced to undergo class switching (42). Thus, hRad51 appears to be regulated in at least two ways: (*a*) transcriptionally, by genes that confer a proliferative potential, as well as by checkpoint signaling pathways that regulate DNA damage responses; and (*b*) at the protein level, where interactions with other molecules leads to distinct cellular localization in hRAD51 nuclear foci. It is possible that hRAD51 regulation may occur at the transcriptional level in a cell type-, cell cycle-, or damage response-coordinated manner. Additional studies will be required to determine the context of the *hRad51* promoter and understand its regulation at the molecular level.

We have determined the chromosomal location of *hRad51* relative to markers used in LOH analysis of human tumors (27). *hRad51* was found to be slightly more centromeric than reported previously (15q14 bordering 15q15) and central to a commonly deleted region in brain metastases from several tumor types and breast carcinomas. However, we did not uncover any mutations in the *hRad51* coding region in a cohort of 42 tumors that displayed LOH in the chromosome 15q14–15 region. In addition, we found no indication that expression of *hRad51* might be down-regulated by hypermethylation of the CpG island in the putative promoter region. It is therefore likely that *hRad51* is not the candidate tumor suppressor gene in these tumors. These results support the notion that hRAD51 may be an essential

gene, a redundant gene, or is dispensable and/or independent of the BRCA1/BRCA2 tumor suppressor pathway(s).

Acknowledgments

We thank Hans-Jürg Alder and the Kimmel Nucleic Acids Facility for oligonucleotide synthesis and sequencing.

References

1. Tlsty, T. D., Briot, A., Gualberto, A., Hall, I., Hess, S., Hixon, M., Kuppuswamy, D., Romanov, S., Sage, M., and White, A. Genomic instability and cancer. Mutat. Res., *337:* 1–7, 1995.
2. Nowell, P. C. The clonal evolution of tumor cell populations. Science (Washington DC), *194:* 23–28, 1976.
3. Loeb, L. A. Mutator phenotype may be required for multistage carcinogenesis. Cancer Res., *51:* 3075–3079, 1991.
4. Dietmaier, W., Wallinger, S., Bocker, T., Kullmann, F., Fishel, R., and Rüschoff, J. Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. Cancer Res., *57:* 4749–4756, 1997.
5. Boveri, T. Zur Frage der Entstehung maligner Tumoren, Fischer. Jena, 1914.
6. Fishel, R., and Kolodner, R. D. Identification of mismatch repair genes and their role in the development of cancer. Curr. Opin. Genet. Dev., *5:* 382–395, 1995.
7. Kolodner, R. Biochemistry and genetics of eukaryotic mismatch repair. Genes Dev., *10:* 1433–1442, 1996.
8. Lengauer, C., Kinzler, K. W., and Vogelstein, B. Genetic instability in colorectal cancers. Nature (Lond.), *386:* 623–627, 1997.
9. Meyn, M. S. Chromosome instability syndromes: lessons for carcinogenesis. Curr. Top. Microbiol. Immunol., *221:* 71–148, 1997.
10. Varon, R., Vissinga, C., Platzer, M., Cerosaletti, K. M., Chrzanowska, K. H., Saar, K., Beckmann, G., Seemanová, E., Cooper, P. R., Nowak, N. J., Stumm, M., Weemaes, C. M. R., Gatti, R. A., Wilson, R. K., Digweed, M., Rosenthal, A., Sperling, K., Concannon, P., and Reis, A. Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. Cell, *93:* 467–476, 1998.
11. Watt, P. M., Hickson, I. D., Borts, R. H., and Louis, E. J. SGS1, a homologue of the Bloom's and Werner's syndrome genes, is required for maintenance of genome stability in *Saccharomyces cerevisiae*. Genetics, *144:* 935–945, 1996.
12. Carney, J. P., Maser, R. S., Olivares, H., Davis, E. M., Le Beau, M., Yates, J. R., Hays, L., Morgan, W. F., and Petrini, J. H. J. The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. Cell, *93:* 477–486, 1998.
13. Baumann, P., and West, S. C. Role of the human RAD51 protein in homologous recombination and double-strand-break repair. Trends Biochem. Sci., *23:* 247–251, 1998.
14. Stürzbecher, H. W., Donzelmann, B., Henning, W., Knippschild, U., and Buchhop, S. p53 is linked directly to homologous recombination processes via RAD51/RecA protein interaction. EMBO J., *15:* 1992–2002, 1996.
15. Scully, R., Chen, J. J., Plug, A., Xiao, Y. H., Weaver, D., Feunteun, J., Ashley, T., and Livingston, D. M. Association of BRCA1 with Rad51 in mitotic and meiotic cells. Cell, *88:* 265–275, 1997.
16. Zhang, H. B., Tombline, G., and Weber, B. L. BRCA1, BRCA2, and DNA damage response: collision or collusion. Cell, *92:* 433–436, 1998.
17. Morrison, D. P., and Hastings, P. J. Characterization of the mutator mutation mut5-1. Mol. Gen. Genet., *175:* 57–65, 1979.
18. Chen, C., Umezu, K., and Kolodner, R. D. Chromosomal rearrangements occur in *S. cerevisiae* rfa1 mutator mutants due to mutagenic lesions processed by double-strand break repair. Mol. Cell, *2:* 9–22, 1998.
19. Barlow, C., Liyanage, M., Moens, P. B., Deng, C-X., Ried, T., and Wynshaw-Boris, A. Partial rescue of the prophase I defects of Atm-deficient mice by p53 and p21 null alleles. Nat. Genet., *17:* 462–466, 1997.
20. Baskaran, R., Wood, L. D., Whitaker, L. L., Canman, C. E., Morgan, S. E., Xu, Y., Barlow, C., Baltimore, D., Wynshaw-Boris, A., Kastan, M. B., and Wang, J. Y. J. Ataxia telangiectasia mutant protein activates c-Abl tyrosine kinase in response to ionizing radiation. Nature (Lond.), *387:* 516–519, 1997.
21. Shafman, T., Khanna, K. K., Kedar, P., Spring, K., Kozlov, S., Yen, T., Hobson, K., Gatei, M., Zhang, N., Watters, D., Egerton, M., Shiloh, Y., Kharbanda, S., Kufe, D., and Lavin, M. F. Interaction between ATM protein and c-Abl in response to DNA damage. Nature (Lond.), *387:* 520–522, 1997.
22. Yuan, Z-M., Huang, Y., Ishiko, T., Nakada, S., Utsugisawa, T., Kharbanda, S., Wang, R., Sung, P., Shinohara, A., Weichselbaum, R., and Kufe, D. Regulation of Rad51 function by c-Abl in response to DNA damage. J. Biol. Chem., *273,* 3799–3802, 1998.
23. Lim, D. S., and Hasty, P. A Mutation in mouse rad51 results in an early embryonic lethal that is suppressed by a mutation in p53. Mol. Cell. Biol., *16:* 7133–7143, 1996.
24. Sonoda, E., Sasaki, M. S., Buerstedde, J. M., Bezzubova, O., Shinohara, A., Ogawa, H., Takata, M., Yamaguchiiwai, Y., and Takeda, S. Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death. EMBO J., *17:* 598–608, 1998.
25. Bishop, D. K., Ear, U., Bhattacharyya, A., Calderone, C., Beckett, M., Weichselbaum, R. R., and Shinohara, A. Xrcc3 is required for assembly of Rad51 complexes *in vivo*. J. Biol. Chem., *273:* 21482–21488, 1998.
26. Liu, N., Lamerdin, J. E., Tebbs, R. S., Schild, D., Tucker, J. D., Shen, M. R., Brookman, K. W., Siciliano, M. J., Walter, C. A., Fan, W., Narayana, L. S., Zhou, Z-Q., Adamson, A. W., Sorensen, K. J., Chen, D. J., Jones, N. J., and Thompson, L. H. XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. Mol. Cell, *1:* 783–793, 1998.
27. Wick, W., Petersen, I., Schmutzler, R. K., Wolfarth, B., Lenartz, D., Bierhoff, E., Hummerich, J., Muller, D. J., Stangl, A. P., Schramm, J., Wiestler, O. D., and von Deimling, A. Evidence for a novel tumor suppressor gene on chromosome 15 associated with progression to a metastatic stage in breast cancer. Oncogene, *12:* 973–978, 1996.
28. Clark, S. J., Harrison, J., Paul, C. L., and Frommer, M. High sensitivity mapping of methylated cytosines. Nucleic Acids Res., *22:* 2990–2997, 1994.
29. Takahashi, E., Matsuda, Y., Hori, T., Yasuda, N., Tsuji, S., Mori, M., Yoshimura, Y., Yamamoto, A., Morita, T., and Matsushiro, A. Chromosome mapping of the human (RECA) and mouse (RecA) homologs of the yeast RAD51 and *Escherichia coli* recA genes to human (15q15.1) and mouse (2F1) chromosomes by direct R-banding fluorescence *in situ* hybridization. Genomics, *19:* 376–378, 1994.
30. Kane, M. F., Loda, M., Gaida, G. M., Lipman, J., Mishra, R., Goldman, H., Jessup, M. A., and Kolodner, R. Methylation of the *hMLH1* promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. Cancer Res., *57:* 808–811, 1997.
31. Cairns, S. S., and Karran, P. Epigenetic silencing of the DNA repair enzyme O^6-methylguanine-DNA methyltransferase in Mex^- human cells. Cancer Res., *52:* 5257–5263, 1992.
32. Bird, A. P. CpG-rich islands and the function of DNA methylation. Nature (Lond.), *321:* 209–213, 1986.
33. Johnson, L. F. G_1 events and the regulation of genes for S-phase enzymes. Curr. Opin. Cell Biol., *4:* 149–154, 1992.
34. Yamamoto, A., Taki, T., Yagi, H., Habu, T., Yoshida, K., Yoshimura, Y., Yamamoto, K., Matsushiro, A., Nishimune, Y., and Morita, T. Cell cycle-dependent expression of the mouse Rad51 gene in proliferating cells. Mol. Gen. Genet., *251:* 1–12, 1996.
35. Basile, G., Aker, M., and Mortimer, R. K. Nucleotide sequence and transcriptional regulation of the yeast recombinational repair gene RAD51. Mol. Cell. Biol., *12:* 3235–3246, 1992.
36. Tashiro, S., Kotomura, N., Shinohara, A., Tanaka, K., Ueda, K., and Kamada, N. S phase specific formation of the human Rad51 protein nuclear foci in lymphocytes. Oncogene, *12:* 2165–2170, 1996.
37. Chen, F. Q., Nastasi, A., Shen, Z. Y., Brenneman, M., Crissman, H., and Chen, D. J. Cell cycle-dependent protein expression of mammalian homologs of yeast DNA double-strand break repair genes Rad51 and Rad52. Mutat. Res., *384:* 205–211, 1997.
38. Inouye, M., and Pardee, A. B. Changes of membrane proteins and their relation to deoxyribonucleic acid synthesis and cell division of *Escherichia coli*. J. Biol. Chem., *245:* 5813–5819, 1970.
39. de Toledo, S. M., Azzam, E. I., Keng, P., Laffrenier, S., and Little, J. B. Regulation by ionizing radiation of CDC2, cyclin A, cyclin B, thymidine kinase, topoisomerase II α, and RAD51 expression in normal human diploid fibroblasts is dependent on p53/p21(Waf1). Cell Growth Differ., *9:* 887–896, 1998.
40. Mercer, W. E., Shields, M. T., Lin, D., Appella, E., and Ullrich, S. J. Growth suppression induced by wild-type p53 protein is accompanied by selective down-regulation of proliferating-cell nuclear antigen expression. Proc. Natl. Acad. Sci. USA, *88:* 1958–1962, 1991.
41. Haaf, T., Golub, E. I., Reddy, G., Radding, C. M., and Ward, D. C. Nuclear foci of mammalian Rad51 recombination protein in somatic cells after DNA damage and its localization in synaptonemal complexes. Proc. Natl. Acad. Sci. USA, *92:* 2298–2302, 1995.
42. Li, M. J., Peakman, M. C., Golub, E. I., Reddy, G., Ward, D. C., Radding, C. M., and Maizels, N. Rad51 expression and localization in B cells carrying out class switch recombination. Proc. Natl. Acad. Sci. USA, *93:* 10222–10227, 1996.
43. Gardiner-Garden, M., and Frommer, M. CpG islands in vertebrate genomes. J. Mol. Biol., *196:* 261–282, 1987.